October 1943 E-604

UNITED STATES DEPARTMENT OF AGRICULTURE
Agricultural Research Administration
Bureau of Entomology and Plant Quarantine

HYDROCYANIC ACID AS A FUMIGANT FOR THE JAPANESE BEETLE

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Introduction

In 1935 Osburn and Lipp 2/ reported that the adult Japanese beetle (*Popillia japonica* Newman) could be killed in a refrigerator car by introducing 6 ounces of hydrocyanic acid and holding the gas in the car for 2 hours at a temperature of 75° F. During the summer of 1937 it was found that this treatment was not always completely effective, particularly when the temperature in the cars was below 75°, and the question was raised by the Division of Japanese Beetle Control how the treatment might be modified to make it effective.

1/ The cooperation of C. W. Stockwell and others of the Division of Japanese Beetle Control and of the officials of the American Cyanamid and Chemical Corporation, the Fruit Growers Express Company, and the Fruit Dispatch Company in this investigation is greatly appreciated. The writers also wish to acknowledge the assistance of C. [illegible] Wible, field aide, in carrying out this investigation during the summers of 1937 and 1938.

2/ Osburn, M. F., and Lipp, J. [illegible] Fumigation of fresh fruit to destroy the adult Japanese beetle. U. S. Dept. Agr. Cir. 373, 29 pp., illus. 1935.

During 1937 and in 1938 an investigation was carried on at Moorestown, N. J., Wilmington, Del., and New York, N. Y., to establish more definitely the relationship between the various factors and the effectiveness of hydrocyanic acid against the adult beetle, and to determine how the treatment might be modified under the different conditions to obtain satisfactory results.

Hydrocyanic Acid Used

The hydrocyanic acid used in this investigation was of the commercial grade, containing 96 to 98 percent of HCN. In most of the experiments sealed cans containing definite amounts of hydrocyanic acid absorbed in fiber discs were used, but in some experiments the desired amount of liquid hydrocyanic acid was measured before being introduced into the refrigerator car.

The sealed cans used in the experiments at Moorestown contained exactly 2 ounces or 6 ounces of HCN. In the experiments at Wilmington, in cooperation with the Division of Japanese Beetle Control, the inspectors used cans containing slightly more than 6 ounces of HCN in order to compensate for the losses that might occur in introducing the material into the refrigerator cars. The fiber discs in the cans were attached at their median points by means of a cord so that a chain of discs could be suspended. Two chains, each with 5 or 6 discs, were in each 6-ounce can. The discs in the cans containing 2 ounces were in one chain or were not attached to one another.

Fumigation Equipment

A 1,000-cubic-foot fumigation chamber at Moorestown, N. J., was used in the major study of the relation between the mortality of the insect and the various factors. This chamber was lined with galvanized iron with all joints soldered, and gaskets were on the door and the ventilating window. The temperature of the chamber was raised by means of a hot-water system, thermostatically controlled, but there was no provision for cooling the chamber. It was necessary to conduct all the experiments at or above the temperature prevailing outside. Fans were provided for circulating the air in the chamber throughout the experimental periods and for removing the gas at the completion of a treatment. This chamber was not absolutely gas-tight but it was believed to be at least as tight as most chambers of its type. The work was done when the velocity of the wind was comparatively low, in order to minimize the factor of leakage.

The experiments in the refrigerator cars were conducted with empty cars that were prepared for loading with fruit or vegetables and with cars loaded with bananas. The average volume of these cars, including the load space and the ice bunkers, was about 2,400 cubic feet. The average air space in a car loaded with bananas was estimated to be about 1,600 cubic feet. A slat floor was laid in the load space. Grills at the top and the bottom of the partitions between the load space and the bunkers permitted air from the load space to circulate through the bunkers.

Although every precaution was taken to try to make these cars gas-tight--sealing the drains from the ice bunkers and securing the hatches and doors firmly in place--the refrigerator cars were less gas-tight than the chamber. No chemical analyses have been made to determine the leakage of gas from the cars, but it was not uncommon to find a very pronounced odor of hydrocyanic acid in the vicinity of a car containing gas, particularly around the doors and hatches.

Minimum Temperatures and Exposures Required for Different Amounts of Hydrocyanic Acid to Kill the Beetles

The experiments with the adult Japanese beetle were conducted during a 6-week period in the summer when this stage was abundant in the vicinity of Moorestown, N. J. This period is too short for extensive experimentation, and the lack of refrigeration in the chamber made it impossible to obtain any information at temperatures below 70° F. when the beetles were present. It was decided, therefore, to conduct as many experiments as possible with the adults and then to continue the experiments throughout the year, using third instars as test insects.

In the experiments with the adults in the fumigation chamber, the beetles were collected in the field from unsprayed trees and shrubs before 7 a.m. of the day they were to be treated, divided into groups of 200, and placed in wire cages. Three cages were used in each experiment. One cage was placed 4 inches above the floor, another 5 feet above the floor, and the third 9 feet above the floor. After the cages were in the chamber, the circulating fans were operated for at least 1 hour before the gas was introduced. At the end of this period a record was made of the temperature, and one or more cans of hydrocyanic acid were opened and the fiber discs were spread in a single layer in a pan on the floor, 8 feet away from the nearest cage. The fans were operated throughout the experimental period. A record was made of the temperature in the chamber at intervals of 15 minutes during the exposure of the beetles to the gas. At the end of the predetermined exposure the cages were removed and placed in an open insectary, where the beetles were kept under observation until their reaction could be determined. As the mortality of unfumigated beetles under these conditions ranged from 0 to 25 percent at the end of 24 hours and then increased rapidly thereafter, the final observations on the fumigated beetles were made 24 hours after their removal from the chamber. After the completion of a treatment the chamber was aerated for at least 18 hours before another experiment was begun.

The experiments with the third instar were conducted in practically the same manner. The larvae were removed from soil and placed without soil in perforated metal cages, which were divided into 100 compartments to prevent the larvae from injuring one another. Three cages with 300 larvae were used in each experiment. After the treatment was completed the larvae were removed from the cages and placed for observation on the surface of moist sifted peat in cross-sectional trays. As the natural mortality of the larvae under these conditions was very low, it was possible to keep the fumigated individuals under observation for a relatively long period--1 to 2 weeks--until the effect of the treatment could be definitely established.

In conducting this investigation, 261 experiments, involving 118,500 fumigated insects and 10,000 unfumigated individuals, were performed in the chamber. The adults were exposed for periods of 0.5 to 3 hours to concentrations of hydrocyanic acid ranging from 2 to 6 ounces per 1,000 cubic feet at temperatures of from 70° to 100° F.; the larvae were exposed for these periods to treatments of from 2 to 10 ounces at temperatures of from 45° to 100°. In these experiments it was possible to maintain the temperature within ± 1° during the treatments, but it was not possible to perform the experiments at fixed intervals between 45° and 100°.

It was evident that there was a changing relationship between the mortality and the temperature and exposure to a given concentration of gas. The mortality varied with the joint effect of temperature and exposure. The joint functional relation between the mortality and these factors was determined according to the procedure outlined by Ezekiel 3/, using the results obtained with 80,400 adults and 38,100 larvae. It was found that the values obtained from the joint functional curves were in very close agreement with the experimentally determined mortalities, the standard error of estimate being about 1 percent. Within the range of the experimental data, the most probable mortalities that would be expected with the different concentrations of hydrocyanic acid and exposures were determined at 5-degree intervals for temperatures ranging from 45° to 100° F. The results are summarized in tables 1 and 2.

As the joint functional values are based on the reactions of several thousands of insects, each value in the tables may be considered as being the most probable mortality of several thousand individuals. It is believed that these values are more reliable and closer to the true mortality than the results of an individual experiment with 300 or 600 individuals. It would be expected, however, that when the tabular value of the treatment is 99 percent there will be many cases in which the treatment will kill all the insects.

3/ Ezekiel, M. Methods of correlation analysis. 427 pp., illus. John Wiley and Sons, New York. 1930.

A careful study of the data in tables 1 and 2 indicates that the mortality curves for the adults and the larvae tend to coincide as they approach 100 percent. As no low mortalities were obtained at temperatures above 70° F. in the identical treatments applied to both stages, it is not possible to determine how these curves would compare at lower levels of mortality. However, since this close relationship exists with mortalities approaching 100 percent in the overlapping experiments at 70° and above, it seemed reasonable to expect that the same relationship would prevail at temperatures below 70°. It would seem proper under these conditions to assume that a treatment that was effective in killing all the larvae at the lower temperatures would be equally effective against the adults. Based on the data given in tables 1 and 2, the minimum exposure and temperature at which 2, 4, 6, and 8 ounces of hydrocyanic acid would be expected to kill practically all the beetles were determined. It would be expected that these treatments would produce 100 percent mortality in many cases, but occasionally 1 percent of the beetles might survive. The minimum requirements for these treatments are presented in table 3.

Table 1.--Mortality of adult Japanese beetles exposed to hydrocyanic acid in a 1,000-cubic-foot fumigation chamber, as determined by the joint functional relation between mortality and the temperature and exposure

Ounces of HCN to 1,000 cubic feet	Exposure in hours	Percent mortality */ at indicated temperature in ° F. 70	75	80	85	90	95	100
2	0.5	68.7	73.6	76.9	79.4	81.3	82.6	83.6
	1	94.8	96.2	97.7	98.2	98.9	99.3	99.6
	1.5	97.7	98.6	99.3	99.6	99.6	100.0	100.0
	2	98.8	99.3	99.8	100.0	100.0	100.0	100.0
	3	100.0	100.0	100.0	100.0	100.0	100.0	100.0
4	0.5	98.2	98.6	98.9	99.1	99.2	99.2	99.2
	1	99.6	99.8	99.9	100.0	100.0	100.0	100.0
	1.5	100.0	100.0	100.0	100.0	100.0	100.0	100.0
	2	100.0	100.0	100.0	100.0	100.0	100.0	100.0
	3	100.0	100.0	100.0	100.0	100.0	100.0	100.0
6	0.5	100.0	100.0	100.0	100.0	100.0	100.0	100.0
	1	100.0	100.0	100.0	100.0	100.0	100.0	100.0

*/ Standard error with the 2-ounce treatment was 1.2 percent and with the 4-ounce treatment it was 1.0 percent.

Table 2.--Mortality of third instars of the Japanese beetle, removed from soil and exposed to hydrocyanic acid in a 1,000-cubic-foot fumigation chamber, as determined by the joint functional relation between mortality and the temperature and exposure

Ounces of HCN to 1,000 cubic feet	Exposure in hours	Percent mortality */ at indicated temperature in ° F.											
		45	50	55	60	65	70	75	80	85	90	95	100
2	1	62.7	74.3	81.3	86.0	89.3	92.3	95.0	--	--	--	--	--
	2	83.7	85.7	95.0	97.3	98.3	99.0	99.7	--	--	--	--	--
	3	92.0	95.0	97.3	98.3	99.3	100.0	100.0	--	--	--	--	--
4	0.5	--	--	--	--	--	--	99.0	99.3	99.7	99.7	99.7	100.0
	1	96.0	97.0	97.7	98.3	99.0	99.0	99.3	99.7	99.7	100.0	100.0	100.0
	1.5	98.0	98.3	99.0	99.3	99.3	99.6	99.6	99.7	100.0	100.0	100.0	100.0
	2	99.0	99.3	99.7	99.7	99.7	99.7	100.0	100.0	100.0	100.0	100.0	100.0
	3	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
6	0.5	--	--	--	--	--	--	99.3	99.7	99.7	99.7	99.7	100.0
	1	98.3	98.7	99.0	99.3	99.3	99.7	99.7	100.0	100.0	100.0	100.0	100.0
	1.5	99.3	99.3	99.7	99.7	99.7	99.7	100.0	100.0	100.0	100.0	100.0	100.0
	2	99.7	99.7	99.7	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
	3	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
8	1	99.0	99.3	99.7	100.0	100.0	100.0	100.0	100.0	--	--	--	--
	2	99.7	99.7	100.0	100.0	100.0	--	--	--	--	--	--	--
	3	100.0	100.0	100.0	100.0	100.0	--	--	--	--	--	--	--
10	1	99.0	99.7	100.0	--	--	--	--	--	--	--	--	--
	2	99.7	100.0	100.0	--	--	--	--	--	--	--	--	--
	3	100.0	100.0	100.0	--	--	--	--	--	--	--	--	--

*/ Standard error with the 2-ounce treatments was 1.1 percent and with the other treatments it was 1.0 percent.

Table 3.--The minimum temperature and exposure required for different concentrations of hydrocyanic acid to kill the adult Japanese beetle

Ounces of HCN per 1,000 cubic feet	Minimum effective exposure in hours	Minimum effective temperature in °F.
2	1	95
	1.5	80
	2	75
	3	65
4	0.5	85
	1	65
	1.5	55
	2	45
6	0.5	70
	1	55
	1.5	45
8	1	45

When hydrocyanic acid is used at the rate of 2 ounces to 1,000 cubic feet, it appears that a minimum temperature of 75° F. is necessary to kill all the adults with a 2-hour exposure. At higher temperatures the period of exposure could be reduced to 1.5 hours at 80° and to 1 hour at 95°. Within a 3-hour exposure the minimum effective temperature for the 2-ounce treatment appears to be 65°.

If it is not practical to prolong the period of treatment for more than 2 hours in the commercial treatments, it would seem necessary to increase the amount of hydrocyanic acid to 4 ounces to 1,000 cubic feet in order to destroy the beetles at temperatures below 75° F. It would be expected that a 2-hour exposure to the 4-ounce treatment would be effective at a minimum temperature of 45°.

Effectiveness in Empty Refrigerator Cars

During the summers of 1937 and 1938 experiments were conducted in empty refrigerator cars at Moorestown, N. J., and Wilmington, Del., with 6 and 12 ounces of hydrocyanic acid per car to determine the effectiveness of the treatments under commercial conditions. In the experiments at Moorestown 11 cages, each containing approximately 200 beetles, were placed throughout the car--at the bottom of each ice bunker, on the floor, 3 feet above the floor, and 6 feet above the floor of the load space; at Wilmington 4 cages containing 50 beetles were placed on the floor between the loading doors. When the cages were in position, a record was made of the temperature in the car and the fumigant was applied. In applying the fumigant 2 men worked together to expedite the operation; one man raised and lowered the hatches of the bunkers, the other handled the hydrocyanic acid. A chilled can of hydrocyanic acid was opened on the roof of the car near a hatch, and a string of fiber discs impregnated with hydrocyanic acid was removed quickly and suspended in the bunker. The can was covered immediately with a fiber cap provided for the purpose, and the hatch was closed and secured in place. Then the remaining string of discs was suspended in the other bunker at the opposite end of the car. At the completion of the predetermined exposure, the hatches were opened and the cages of beetles were removed from the car and taken to the insectary at Moorestown for observation. The effectiveness of the treatments in 31 empty refrigerator cars is summarized in table 4.

It is evident that there was considerable variation in the effectiveness of the treatments in the different cars or applied at different times in the same car. In some cases with the 6-ounce treatment complete mortality was obtained at temperatures well below 75° F. or with exposures of less than 2 hours, but the results appear to demonstrate that practically complete destruction of the beetles can be expected consistently with this treatment only when the average temperature is 75° or above and the exposure is 2 hours.

The results obtained with the 6-ounce treatment for 3 hours were no better than those with the 2-hour treatment. In fact, in the tests of the 6-ounce treatment for 3 hours at 72° and 73° F. the rates of mortality were abnormally low. It appears that the refrigerator cars are not sufficiently gastight to be used for prolonged exposures.

The data obtained with the 12 -ounce treatment were rather meager but they seem to indicate that better results can be obtained with this treatment than with the 6-ounce treatment.

Table 4.--Effectiveness of hydrocyanic acid against the adult Japanese beetle in empty refrigerator cars

Ounces of HCN per car	Exposure in hours	Average temperature in °F.	Location of car	Number of beetles in car	Percent mortality
6	0.5	80.0	Moorestown, N.J.*	2,175	60.0
	1	75.8	Moorestown, N.J.	2,209	83.2
		77.8	Moorestown, N.J.	2,184	99.1
		78.5	Moorestown, N.J.	2,214	90.4
		82.5	Moorestown, N.J.	2,112	99.1
		88.5	Moorestown, N.J.	2,201	97.5
	1.5	73.5	Moorestown, N.J.	2,200	100.0
		79.0	Moorestown, N.J.	2,097	96.1
		81.0	Moorestown, N.J.	2,198	99.9
	2	48.0	Wilmington, Del.	200	81.0
		52.0	Wilmington, Del.	200	89.0
		52.0	Wilmington, Del.	200	85.5
		52.0	Wilmington, Del.	200	90.5
		52.0	Wilmington, Del.	200	100.0
		53.0	Wilmington, Del.	200	100.0
		54.0	Wilmington, Del.	200	100.0
		55.0	Wilmington, Del.	200	60.0
		55.0	Wilmington, Del.	200	72.0
		55.0	Wilmington, Del.	200	100.0
		56.0	Wilmington, Del.	200	99.0
		57.0	Wilmington, Del.	200	99.0
		57.0	Wilmington, Del.	200	100.0
		58.0	Wilmington, Del.	200	66.5
		58.0	Wilmington, Del.	200	94.0
		60.0	Wilmington, Del.	200	77.5
		60.0	Wilmington, Del.	200	94.5
		62.0	Wilmington, Del.	200	95.0
		63.0	Wilmington, Del.	200	82.0
		66.0	Wilmington, Del.	200	97.0
		67.0	Wilmington, Del.	200	100.0
		70.0	Wilmington, Del.	200	97.0
		70.0	Wilmington, Del.	200	[illegible]
		71.0	Wilmington, Del.	200	99.0
		71.0	Moorestown, N.J.	2,200	99.9

Table 4 - cont.

Ounces of HCN per car	Exposure in hours	Average temperature in °F.	Location of car	Number of beetles in car	Percent mortality
6	2	72.0	Wilmington, Del.	200	98.5
		72.0	Wilmington, Del.	200	96.0
		72.0	Wilmington, Del.	200	98.5
		72.0	Wilmington, Del.	200	97.0
		73.0	Wilmington, Del.	200	96.0
		73.0	Wilmington, Del.	200	98.5
		74.5	Moorestown, N.J.	2,200	100.0
		75.0	Moorestown, N.J.	2,200	100.0
		79.0	Moorestown, N.J.	2,201	99.7
		79.0	Moorestown, N.J.	2,200	100.0
		82.0	Moorestown, N.J.	2,200	100.0
	3	72.0	Moorestown, N.J.	2,185	58.5
		73.0	Moorestown, N.J.	1,106	88.8
		75.5	Moorestown, N.J.	2,200	100.0
		79.5	Moorestown, N.J.	2,200	100.0
		84.0	Moorestown, N.J.	2,199	98.3
12	1	74.0	Moorestown, N.J.	2,200	100.0
		80.0	Moorestown, N.J.	2,201	99.0
	1.5	90.5	Moorestown, N.J.	2,005	99.9
	2	83.0	Moorestown, N.J.	2,200	100.0
		87.0	Moorestown, N.J.	2,200	100.0

* At Moorestown, N. J., cans containing 6 ounces of HCN were opened by the investigators and half of the charge was introduced into each bunker. At Wilmington, Del., cans containing slightly more than 6 ounces of HCN, to compensate for loss during handling, were used by inspectors of the Division of Japanese Beetle Control.

All tests at Moorestown, N. J., were conducted in the same car. The tests at Wilmington were conducted in 30 different cars, only one treatment being applied to a car.

Effectiveness in Refrigerator Cars Loaded With Cold, Wet Bananas

Osburn and Lipp [4] found that an application of even 1.5 pounds of hydrocyanic acid in a refrigerator car loaded with dry green bananas caused no injury to the fruit. Records show that over 100 carloads of bananas in this condition have been fumigated under commercial conditions, 6 ounces being used per car, without causing damage to the fruit. The situation was quite different with refrigerated fruit, which quickly became covered with condensed atmospheric moisture during the process of transferring it from the refrigerated rooms on the ship to the cars. Extensive injury was reported as resulting from the 6-ounce treatment under these conditions. As the bananas normally occupy about one-third of the load space in a car, and the nature and the packing of the fruit are such that the diffusion of the gas was not a limiting factor, it appeared that if the amount of hydrocyanic acid could be reduced to 5 ounces per car the injury to the fruit might be largely prevented.

One hundred cages, each containing approximately 50 beetles, were distributed among the fruit during the process of loading 20 cars with wet, cold, green bananas at the pier in New York, N. Y. The temperature of the air in the cars during the process of loading was about 82° F., but when the doors were closed it dropped rapidly to about 62°. After the doors were closed, 5 ounces of hydrocyanic acid were introduced into the ice bunkers of each car--2.5 ounces per bunker. The liquid was measured and poured through a long tube into vaporizing pans suspended in the bunkers. At the end of 2 hours the hatches of the cars were opened. The cages of beetles were removed immediately from 10 of the cars, but the remainder of the cages were not removed until 2 hours later, after the cars had been transferred on floats from New York to Weehawken, N. J. The beetles were then taken to Moorestown, N. J., for observation. The mortality obtained in the different cars is summarized in table 5.

[4] Loc. cit.

Table 5.--Effectiveness of hydrocyanic acid against the adult Japanese beetle in refrigerator cars loaded with wet, green bananas at New York, N. Y.

Ounces of HCN per car	Exposure in hours	Average temperature in °F.	Period after fumigation when beetles were removed from car	Number of beetles in car	Percent mortality
5	2	58	Immediately	256	46.5
		58		248	55.6
		60		251	68.1
		60		248	72.2
		62		249	74.7
		62		256	80.5
		62		247	84.6
		62		256	85.6
		64		246	85.8
		64		251	88.4
	Average	61.2	Total	2,508	Ave. 74.2
		59	Two hours after opening the ventilators	250	52.8
		60		243	67.9
		62		286	71.0
		62		247	76.9
		62		242	77.3
		62		254	79.5
		62		252	81.0
		62		250	83.2
		64		250	84.4
		65		252	93.7
	Average	62.0	Total	2,526	Ave. 76.7

The mortality ranged from 46.5 to 93.7 percent. There was no evidence that leaving the beetles for 2 hours in the cars after the hatches were opened increased the effectiveness of the treatment. The reports from the fruit company indicated that the damage with the 5-ounce treatment was just as extensive as with the 6-ounce treatment.

It seemed to be impossible to kill all the beetles in cars of wet, cold, green bananas without causing serious injury to the fruit. The use of hydrocyanic acid under these conditions is unsatisfactory.

Summary and Conclusions

In 1937 and 1938 an investigation was made of hydrocyanic acid as a fumigant for the Japanese beetle (Popillia japonica Newman) to establish more definitely the relation between the mortality of the insect and the concentration of the gas, the duration of the treatment, the temperature, diffusion, and other factors, and to determine what treatments should be applied to refrigerator cars to assure the destruction of the insect. The experiments were conducted with 184,500 insects in a 1,000-cubic-foot chamber and in standard refrigerator cars at Moorestown, N. J., Wilmington, Del., and New York, N. Y., with various concentrations of gas and exposures up to 3 hours at temperatures ranging from 45° to 100° F.

When leakage, absorption, and diffusion were not important factors, as in the fumigation chamber, the results indicate that complete destruction of the beetles can be expected with hydrocyanic acid at the rate of 2 ounces to 1,000 cubic feet of space with an exposure of 1 hour at 95°, 1.5 hours at 80°, 2 hours at 75°, and 3 hours at 65° F. When used at the rate of 4 ounces to 1,000 cubic feet, an exposure of 0.5 hour would be required at 85°, 1 hour at 65°, 1.5 hours at 55°, and 2 hours at 45° F. The 6-ounce treatment would require 0.5 hour at 70°, 1 hour at 55° F, and 1.5 hours at 45°, and the 8-ounce treatment 1 hour at 45° F.

The results indicate that the introduction of 6 ounces of hydrocyanic acid into an empty refrigerator car can not be depended upon to destroy all the beetles within a 2-hour period when the temperature is less than 75° F. As it is impractical to prolong the treatment of these cars for more than 2 hours under commercial conditions, the treatment can be made effective at temperatures below 75° F. only by increasing the amount of the fumigant. It is suggested that at temperatures between 45° and 75° F. the hydrocyanic acid be used in empty refrigerator cars at the rate of 12 ounces per car.

It was not possible to kill all the beetles in refrigerator cars loaded with wet green bananas without causing serious damage to the fruit.